PB88-185558

Fluorometric Flow Injection Determination of Aqueous Peroxides at Nanomolar Level Using Membrane Reactors

Texas Tech Univ., Lubbock

Prepared for

Environmental Protection Agency
Research Triangle Park, NC

1986

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1. REPORT NO. EPA/600/J-86/456	2.
3. R PB88-185558	
4. TITLE AND SUBTITLE FLUOROMETRIC FLOW INJECTION DETERMINATION OF AQUEOUS PEROXIDES AT NANOMOLAR LEVELS USING MEMBRANE REACTIONS (Journal Version)	5. REPORT DATE 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) H. Hwang and P. K. Dasgupta	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Chemistry, Texas Tech University, Lubbock, Texas 79409	10. PROGRAM ELEMENT NO. N104/C/05/05 - 5052 (FY-88)
	11. CONTRACT/GRANT NO. CR812366
12. SPONSORING AGENCY NAME AND ADDRESS Atmospheric Sciences Research Laboratory-RTP, NC, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/09

15. SUPPLEMENTARY NOTES

Analytical Chemistry, 58(7):1521-1524, June 1986

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 6
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE PC $9.95/MF $6.95

EPA Form 2220-1 (9-73)

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EPA/600/J-86/456
JOURNAL ARTICLE

Fluorometric Flow Injection Determination of Aqueous Peroxides at Nanomolar Level Using Membrane Reactors

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A flow injection system based on the *p*-hydroxyphenylacetate–peroxide–peroxidase reaction allows the simultaneous determination of H_2O_2 and CH_3HO_2 at 50 samples/h with an LOD of 0.1 μg/L (3 nM) H_2O_2. A pressurized porous PTFE membrane reactor introduces the enzyme, and the pH of the flow stream is altered by introducing NH_3 through a nonporous cation exchange membrane reactor. Excellent reproducibility, precision, and sensitivity are achieved with the membrane reactors and an inexpensive filter fluorometer with a monochromatic excitation source. Low levels of residual peroxide in water are ubiquitous; such levels return soon after the H_2O_2 is catalytically removed.

Trace determination of aqueous peroxides is of considerable importance in atmoshperic and clinical applications as the number of publications in recent issues of this journal alone (*1–10*) indicates. In atmospheric applications, the requisite sensitivity limits the choice to luminescence methods. Although a sensitive chemiluminescence method has been recently described (*11*), the photoluminescence (fluorescence) procedure involving the oxidation of nonfluorescent *p*-hydroxyphenylacetic acid (PHPA) to its fluorescent dimer by peroxides mediated by the enzyme peroxidase (donor: hydrogen peroxide oxidoreductase EC 1.11.1.7) remains a sensitive interfce-free attractive procedure (*2, 3, 12*). The oxidation product of PHPA fluoresces optimally only above pH 10, while the enzyme works best at a pH of 5.5 (*3, 12*). Consequently, a continuous flow analysis system based on this reaction has at least three flow channels: a carrier (water) channel in which the sample is placed via a loop injector, a PHPA–peroxidase (pH ~5.5) channel which is merged with the water flow channel past the injector, and a base introduction channel to raise the pH of the stream after the reaction has been allowed to occur. More typically, to improve day-to-day reproducibilities of blank levels and calibration slopes (*12*), PHPA and peroxidase are delivered from separate reservoirs, thus requiring a total of four flow channels (*3*).

In any type of continuous flow analysis, detector noise increases with increasing number of pumped channels and deteriorates the ultimately attainable LOD due both to flow noise and mixing inhomogeneity. In this paper, we describe the use of novel active and passive membrane reactors for the introduction of peroxidase and a base, respectively, such that only two pumped channels are required.

An active membrane reactor employs a small-bore, microporous, tubular membrane segment that constitutes part of the flow stream and which is immersed in the desired reagent. The reagent is introduced into the main flow stream through the membrane walls by pressure, typically pneumatic (*13*). The parameters that govern the reagent introduction rate include membrane pore size, tortuosity, surface porosity, thickness, available surface area, and superincumbent pressure. For any given reagent concentration and membrane type, the introduction rate is most conveniently controlled by choosing the length of the membrane and the superincumbent pressure. In this work, we use a PTFE-filament-filled porous PTFE membrane; this reactor is sufficiently inert to permit introduction of highly corrosive reagents if desired. The filament-filled configuration (*14*) reduces dispersion, improves radial mixing by reduction of the available annular space, and lends structural support to the membrane assembly.

A passive membrane reactor differs from an active membrane reactor in that the membrane is essentially nonporous and no pressure is applied on the reagent; the reagent permeates into the flow stream due to the existing concentration differential (*13, 15*). In the present application, we use a perfluorosulfonate cation exchanger membrane tube that is immersed in concentrated NH_4OH. The cation exchange membrane permits inward transport of ammonia both as the uncharged molecule and by cation exchange with NH_4^+; the Donnan barrier due to the negatively charged membrane matrix (*16*) prevents the loss of the anionic fluorophore of interest. Membrane type, thickness, available surface area, and reagent concentration govern reagent introduction rate; no volumetric dilution occurs with a passive reactor. It is shown that in combination with an inexpensive filter fluorometer employing a line excitation source, the membrane reactor flow-injection analysis (FIA) system permits an LOD of ≤0.1 ppb (3×10^{-9} M) H_2O_2.

EXPERIMENTAL SECTION

Sources, preparation, and purification of reagents and standardization of calibrants have been previously described (*2, 12*). The flow schematic of the system is shown in Figure 1. One channel of the peristaltic pump, A (Minipuls 2, slow-speed drive module, Gilson Medical Electronics, Middleton, WI, pump setting 990), is used to pump carrier water at 2.40 mL/min (3.16-mm-i.d. PVC pump tubing) through a packed reactor, B (PTFE, 6 cm active length, 4.5 mm i.d., glass wool plugs at each end), containing granular MnO_2 (Mallinckrodt). The purpose of this reactor is to remove any residual peroxide in the reagent water (*2*). The water flows through a loop-type PTFE rotary injection valve, C (Rheodyne type 50, with 0.8-mm connecting passages, Cotati, CA), equipped with a 750-μL loop. The sample is loaded in the loop through valve L, which can be positioned either for direct transport of the sample to injection valve C or via passage through reactor K (PTFE, 9 cm active length, 4.5 mm i.d.) packed with granular MnO_2. The second channel of pump A pumps the PHPA reagent at a flow rate of 0.60 mL/min (1.00-mm-i.d. PVC tubing). This reagent is composed of 4.0 g of recrystallized PHPA and 0.30 g of Na_2EDTA per liter of solution, adjusted to pH 5.5 with NaOH. This reagent and others described below were all filtered to remove any suspended particulate material before use. The PHPA reagent is mixed with the water stream at mixing tee D, subsequent to the sample introduction point. After a knotted mixing line (*2, 12*), E (50-cm, 0.8-mm-i.d. PTFE tube), the stream enters the pressurized porous membrane reactor, F, which introduces the enzyme radially. The peroxidase-containing flow stream then proceeds through a second knotted mixing line and a delay coil, G (1-m, 0.8-mm-i.d. PTFE tube), which together provides a reaction time of ~15 s. The flow stream then passes through the passive membrane reactor, H, where ammonia is radially introduced to raise the pH to ~10 and flows into a fluorescence detector (Fluoromonitor III, Laboratory Data Control, Riviera Beach, FL) equipped with a Cd lamp, 30-μL flow through cell, 326-nm emission, and 370-nm (high-pass) excitation filters.

Membrane reactor F is made of a 1.5 cm length of a porous PTFE membrane tubing (Gore-Tex TA 001, mean pore size 2 μm,

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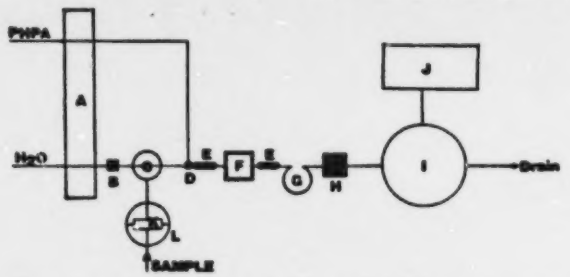

Figure 1. Analytical system schematic: (A) peristaltic pump, (B) MnO_2 reactor to remove residual peroxide from carrier water, (C) PTFE loop injector valve, (D) mixing tee, (E) knotted mixing line, (F) pressurized porous PTFE membrane reactor for introduction of peroxidase, (G) PTFE delay coil, (H) cation exchange membrane reactor for the introduction of ammonia, (I) filter fluorometer with Cd source, (J) recorder, (K) MnO_2 reactor for differentiation of H_2O_2 and organic peroxides, (L) selector valve to place K in- and off-line.

surface porosity 50%, 1 mm i.d., 1.8 mm o.d., W. L. Gore and Associates, Elkton, MD) filled with a 0.8-mm-diameter PTFE filament to reduce the dead volume and to lend structural support to the membrane assembly. PTFE tubes (24 gauge) are forcibly inserted into the terminal ends of the membrane tube for making fluid connections. No special measures are necessary to make the connections leakproof, since a higher external pressure is present during operation. The membrane segment is completely immersed in a solution of peroxidase (0.4 mg/mL) in a 0.05 M phosphate buffer (pH 5.8), contained in a wide-mouth, screw-capped polypropylene bottle of 100-mL capacity. The reactor inlet/outlet lines exit through a silicone rubber stopper, while a central hole admits a tube supplying the pressurizing gas. A large hole is bored out in the original screw-cap to allow passage of the tubes. Screwing in the cap with the stopper in place allows pneumatic pressurization up to 20 psi while the stopper is safely held in place; substantially greater pressures are not safe for the bottle itself. The rate of reagent introduction into the inner flow stream can be controlled by controlling the superincumbent pressure. The exact pressure required is of course dependent on the system back pressure past the reactor. Compressed house air was admitted to the reactor bottle via an inexpensive single-stage pressure regulator; the optimum reagent introduction rate in the present system was determined to occur at a pressure of ~12 psi, corresponding to a reagent flow rate of ~75 μL/min. It should be noted that air pressurization in excess of 20 psi generally creates detector problems associated with bubble formation; helium can be used satisfactorily at higher pressures in an appropriately protected reactor vessel.

The passive membrane reactor, is comprised of a 6.5 cm length of a perfluorosulfonate cation exchange membrane tubing (Nafion 811x, ~1-mm wet i.d., ~75-μm wall thickness, Perma-Pure Products, Toms River, NJ) filled with a 0.8-mm-diameter PTFE filament. Connections to the filament-filled Nafion membrane were made as previously described (*14*). The membrane assembly was completely immersed in a stoppered bottle of 25-mL capacity containing concentrated NH_4OH. The membrane length chosen allowed a sufficient rate of ammonia introduction to raise the effluent pH to ~10 during operation.

Three different detectors were evaluated for the present application: a spectrofluorometer (Baird NOVA, 150-W Xe exciting source with grating monochromators on both excitation and emission sides), a filter fluorometer with a Cd line source as described earlier, and a hybrid instrument that employs a grating monochromator on the excitation side and a high-pass filter (370 nm) on the emission side (50-W D_2 source, Kratos FS 970). The optimum excitation wavelength for the fluorophore of interest is 329 nm, and emission maximum occurs at 412 nm (*12*). The available excitation energy at the wavelength of interest is lowest for the D_2 source; however, this is partly compensated by the excellent emitted light collection efficiency of this instrument.

RESULTS AND DISCUSSION

Typical data from the system are shown in Figure 2. The precision at levels above 10 μg/L of H_2O_2 is typically below

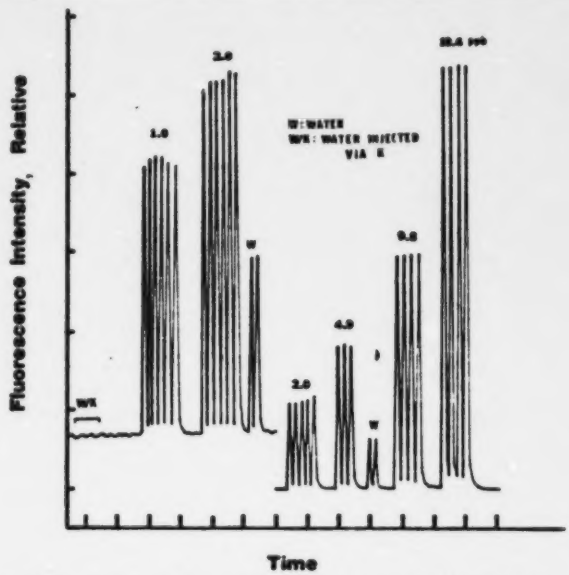

Figure 2. Typical system performance. The concentrations indicated are nominal values in micrograms per liter; the gradations on the time axis are 6 min apart. The left set is run at increased detector sensitivity. Level of residual peroxide in water is always significant. See text for details.

2% relative standard deviation. At lower levels, the relative standard deviation increases, primarily due to the omnipresent and variable water blank. H_2O_2 present in our laboratory reagent water (which exceeds all ASTM Type I specifications) typically measures between 0.3 and 2 μg/L and returns to a steady-state value within this range shortly after the H_2O_2 is catalytically destroyed, as long as dissolved oxygen is present. The reappearance of the H_2O_2 signal is accelerated by visible and long-wave UV light and less so by short-wave UV light. Water saturated with 0.95 atm O_2 produces an order of magnitude greater steady-state level of H_2O_2 than air-saturated water. A typical water signal and its disappearance when injected through the MnO_2 reactor is shown in Figure 2.

Because the blank values are obviously very much higher than the LOD, the LOD cannot be computed directly from the S/N ratio of a low-level nominal standard, e.g., 1 μg/L of H_2O_2. The residual peroxide present in the water to make up such a standard may equal or exceed the nominal value. For the particular series of signals shown in Figure 2, the results can be interpreted as those of a standard addition method, and the blank is computed to contain an H_2O_2 concentration of 1.8 μg/L. Based on the base-line noise levels as shown in this figure, and the guidelines of the American Chemical Society Committee on Environmental Improvement (*18*), the detection limit is computed to be 0.1 μg/L. The attainment of this LOD is derived from the use of the membrane reactors as well as the line-source fluoroescence detector.

Experiments were conducted with different combinations of enzyme concentration in the reactor solution and the volumetric introduction rate (a function of the pneumatic pressure), which results in the same overall rate of reagent introduction. Because excellent mixing was observed under all conditions (in terms of the detector noise level), the combination of a high reagent concentration in the reactor and a very small volumetric introduction rate was chosen; dilution of sample is thus minimized. It should be noted that mixing inhomogeneity in an analogous pumped, tee-mixed, system is a serious problem when the flow rate of one stream is more than 45-fold that of the other, as in this work. In lieu of a pumped channel of PHPA, the following were attempted: (a)

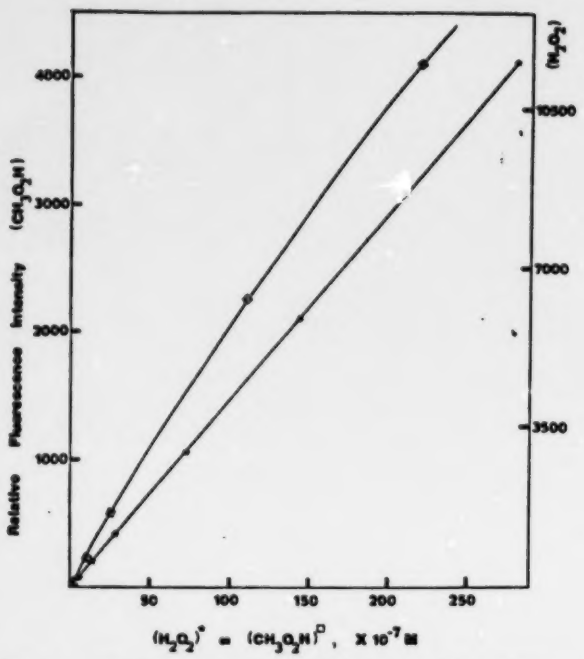

Figure 3. Calibration plots for H_2O_2 and CH_3HO_2. For H_2O_2, the system obeys a linear equation; for CH_3HO_2 the molarity can be calculated from a quadratic equation: $Y = (4.27 \times 10^{-9})S + (2.72 \times 10^{-3})S^2 + 3.94 \times 10^{-4}$, where Y is the molarity and S is the signal in relative fluorescence units, above Y = 100 nM, below which the response is linear.

introduction of a mixed PHPA–peroxidase reagent through the active membrane reactor, (b) introduction of PHPA by an independent active membrane reactor preceding the one used for enzyme introduction, and (c) introduction of PHPA through an independent passive membrane reactor. A mixed PHPA–peroxidase reagent leads to increasing blanks and varying calibration slopes as the reagent ages, similar to that observed in other work (*12*); very exacting pressure regulation is necessary on both the active reactors to maintain the desired flow rates to practice b; and dialysis or anion exchange membrane tubing available to us did not lead to a convenient reagent introduction rate for facile practice of c. Additionally, concentrated and/or saturated reagent solutions are generally desirable for prolonged use in a passive membrane reactor before the reagent concentration is significantly decreased and replenishment is necessary. Unfortunately, PHPA displays a relatively limited solubility in water. In the passive membrane reactor used for the introduction of ammonia, 20 mL of concentrated NH_4OH lasts several days.

Our experience with these membrane reactors also indicates that they exert a pulse-dampening action, presumably due to their elastomeric nature, and thus lower the base-line noise.

In overall performance, the filter fluorometer displayed a S/N at least 2-fold better than the hybrid instrument, which marginally outperformed the spectrofluorometer. The close match between the principal emission line of Cd lamp (326 nm) with that of the optimal excitation wavelength provides efficient high-energy excitation, while the cutoff emission filter strongly discriminates against stray exciting radiation, and this allows the least expensive and most compact (of considerable importance in eventual intended airborne application of the system) instrument to perform the best.

Figure 3 shows that the linearity of response to various levels of H_2O_2 is excellent over a large dynamic range. The deviation from linearity at 2 ppm (6×10^{-5} M) H_2O_2 is only ~5%. Compared to H_2O_2, the system response to CH_3HO_2 is linear over a smaller dynamic range. The response factor for CH_3HO_2 is also significantly lower, 0.57 for the lower, linear portion, relative to H_2O_2. Both the linearity and response factor can be improved by increasing the reaction time. However this leads to greater dispersion and lower throughout rates, and the "improvement" is only aesthetic in nature; the CH_3HO_2 concentration is predicted with very good confidence (correlation coefficient > 0.9999) by a second-order equation (line shown in Figure 3).

Table I. Peroxide Content of Snow Samples, Lubbock, TX

date	H_2O_2 content, μg/L (±SD)[a]
12/11/85	33.5 ± 2.3
12/12/85	146 ± 3.2
12/14/85	
0700–0900 h	537 ± 10.8
0900–1100 h	361 ± 5.3
1100–1400 h	214 ± 2.1
1400–1700 h	137 ± 1.4

[a]Triplicate injections for each sample.

The nested loop injection system (*2*) was introduced for the differential analysis of H_2O_2 and organic peroxides. However, this arrangement hampered trace analysis in the present system due to pressure surges accompanying the introduction and removal of the packed MnO_2 reactor in the main flow stream by valve actuation. The present system therefore uses a valve-switchable MnO_2 reactor in the sample loading line, which as before, allows the determination of organic peroxides only when the reactor is in-line, while total peroxides are measured with the reactor off-line. Two sequential injections are required; however, the valve can be easily automated, and the overall throughput rate (50/h) is significantly better than the nested loop system. The reactor dimensions for the present system were tailored so that for manual loading through the reactor H_2O_2 was completely destroyed while CH_3HO_2 showed no perceptible decrease over a 3-fold range of reactor residence time (volumetric rate of sample loading). For the reactor described, these conditions are met over a loading rate from 0.20 mL/s (deliberately slow loading) to 0.62 mL/s (the highest loading rate attainable with full manual pressure on a 10-mL syringe). The reproducibility of the system in terms of blank level and calibration slope on consecutive days with the same batch of reagents is better than ±1%. Some representative results of analyses of precipitation samples are shown in Table I.

Registry No. H_2O_2, 7722-84-1; CH_3HO_2, 690-02-8; PTFE, 9002-84-0; MnO_2, 1313-13-9; H_2O, 7732-18-5; *p*-hydroxyphenylacetate, 156-38-7; peroxide, 14915-07-2; peroxidase, 9003-99-0.

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RECEIVED for review August 19, 1985. Accepted January 14, 1986. This research was supported by the U.S. Environmental Protection Agency through R810894-010 and CR812366-01-0. However, this report has not been subject to review by the Environmental Protection Agency and therefore does not necessarily reflect the views of the Agency, and no official endorsements should be inferred.

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